

Effects of Nucleotide-Inhibitor Sequence on the Binding of E-box DNA to Mitf (Microphthalmia-associated Transcription Factor) in the Protein Chip

Eun-Young Kwak · Jung-Sun Han · Hyang-Bok Lee · Jung-Hyun Shin* · Eun-Ki Kim†

Department of Biological Engineering, Inha University, Incheon 402-751, Korea

*Department of dermatology, College of Medicine, Inha University

Abstract

For the high-throughput-screening system (HTS) of depigmenting agents by a protein chip, effects of oligonucleotide-inhibitor sequence on the binding of Mitf protein to E box of MC1R was investigated. The sequence of oligonucleotide-inhibitor affected the binding of the target DNA to Mitf, depending on the location of the sequence variation in the inhibitor nucleotide. The oligonucleotide-inhibitor that changed the CATGTG sequence showed no enough inhibition of the target DNA to Mitf, whereas significant inhibition was observed when the sequence outside the CATGTG was varied. This result indicated that CATGTG is crucial sequence for the binding of Mitf to E-box which initiates the transcription of pigmenting genes.

References

1. T. Ellenberger, D. Fass, M. Arnaud, and S.C. Harrison, Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer, *Genes. Dev.*, **8**, 970 (1994).
2. H. Aoki and O. Moro, Involvement of microphthalmia-associated transcription factor (MITF) in expression of human melanocortin-1 receptor (MC1R), *Life Sci.*, **71**, 2171 (2002).
3. J. M. Jung, Y. B. Shin, M. G. Kim, H. S. Ro, H. T. Jung, and B. H. Chung, A fusion protein expression analysis using surface plasmon resonance imaging, *Anal. Biochem.*, **330**, 251 (2004).
4. J. Linnell, R. Mott, S. Field and I. A. Udalova, Quantitative high-throughput analysis of transcription factor binding specificities, *Nucleic Acids Res.*, **32(4)**, (2004).
5. O. Moro, R. Ideta, and O. Ifuku, Characterization of the promoter region of the human melanocortin-1 receptor(MC1R) gene, *Biochemical and Biophysical Research Communications*, **262**, 452 (1999).